15. (New) A glycosylated human antithrombin III which is produced in mammary gland of a non-human transgenic mammal, wherein the antithrombin III comprises a monosaccharide composition which comprises GalNAc.

16. (New) The glycosylated human antithrombin III of claim 15, wherein the antithrombin III further comprises a monosaccharide composition which is partially sialyated.

17. (New) The glycosylated human antithrombin III of claim 15, wherein the antithrombin III further comprises a monosaccharide composition comprising sialic acid which includes NGNA.

18. (New) The glycosylated human antithrombin III of claim 16 or 17 wherein the antithrombin III has a faster clearance time than plasma derived antithrombin III.

REMARKS

Claims 1, 2, 6, 7, 9, 10 and 11-18 are pending in the present application. Claims 1, 2, 6, 7, 9, 10 and 11 have been amended. Claims 3-5, and 8 have been cancelled without prejudice. However, the amendments to and/or cancellation of the claims were made solely to expedite prosecution of the present application. No new matter has been added.

Double Patenting Rejection of Claims 1-11

Claims 1-11 are rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-8 of U.S. patent No. 5,843,705. According to the Examiner,

> [a]lthough the conflicting claims are not identical, they are not patentably distinct from each other because the applicant claims a transgenically produced antithrombin III and method for producing antithrombin III in mammalian milk, comprising transgenic mammal that expresses a transgene which encodes antithrombin III with a monosaccharide composition which includes GalNAc or Fuc, GlcNAc, Gal, Man, NANA/NGNA having one of its glucosylation sites

comprising an oligomannose and/or hybrid oligosaccharide structures; collecting milk; and isolating human antithrombin III from milk.

A Terminal Disclaimer is being submitted herewith, thereby overcoming this rejection.

Rejection of Claims 1-11 under 35 U.S.C. §112, first paragraph

Claims 1-11 are rejected under 35 U.S.C. §112, first paragraph, "because the specification, while being enabling for antithrombin III having a specific glycosylation pattern and obtained from transgenic goat milk, does not reasonably provide enablement for any antithrombin III having any glycosylation differences and obtained from a transgenic mammal." In particular, the Examiner states that

> [t]he claims, as written, read on any animal model. However, the specification fails to provide any teachings or guidance with regards to the generation of any other animal model, other than transgenic goat which would predictably produce the instantly claimed monosacchride composition. The art of transgenics is not a predictable art with respect to transgene behavior. Without evidence to the contrary, transgene expression in different species of transgenic non-human animals is not predictable and varies according to the particular host species. The observation is further supported by Mullins et al. (Journal of Clinical Investigations, 1996) who report on transgenesis in rat and larger animals. Mullins et al. state that "a given construct may react very differently from one species to another." See page S39, Summary. Cole et al (1994) J. of Cellular Biochemistry Suppl.. Vol. 0 (18D) p 265 disclose that NGNA and NANA acids were found on both therapeutic proteins (antithrombin III and LA-tPA), NGNA therefore appears to be a function of expressing the proteins in goats. Given such species differences in the expression of a transgene, one of skill in the art would have been required to undergo undue experimentation to determine which promoter, enhancer, intron, exon, and transgene construct would produce the desired phenotype in <u>any and all</u> animals.
>
> [a]s a second issue, while the state of the art of transgenics is such that one of skill in the art would be able to produce transgenic goat comprising antithrombin III transgene of interest wherein antithrombin III secreted by goat milk has a specific glycosylation pattern inherent to the host species, it is not predictable that the broad glycosylation pattern of the expressed antithrombin III of the claimed invention would be produced by said goat.
>
> The intention of the animal model, as defined in the specification of the instant application, is for transgenically producing antithrombin III in goats' milk comprising monosaccharides having a specific glycosylation pattern. The claims

read on any glycosylation pattern of ATIII that differs from that found in human plasma, but the specification only teaches specific glycosylation patterns of goat produced antithrombin III. Given such a distinction in glycosylation pattern to host in the expression of antithrombin III, it would require undue experimentation to generate a general model that exhibits all the glycosylation pattern seen in any transgenic mammal or any glycosylation pattern of antithrombin III. It is standardly and well known in the art that glycosylation patterns are a function of the host cell in which the protein product is translated and post-translationally modified by the host enzymes. There is insufficient objective evidence provided to indicate that the numerous embodiments of different glycosylation patterns now claimed would be predictably obtainable from a goat host or any other host. In Drohan review (1997), "The past, present and future of transgenic bioreactors" disclosed that proteins have few posttranslational modifications, mainly removal of any signal peptide and N-linked glycosylation. It appears that cleavage of signal peptides and the addition of carbohydrate chains at multiple sites to protein precursors in the mammary gland are performed adequately. Drohan further state that the carbohydrate composition and structure of transgenic proteins may differ from that of their human counterparts. Drohan continue further by disclosing the site-specific addition of oligomannose to a specific asparagines residues of recombinant ATIII was also observed in the goat mammary gland. Drohan concludes that these species and protein- specific glycosylation patterns may affect therapeutic efficacy, binding to cellular receptors and clearance of recombinant proteins in patients. The specification is only enabling for a transgenic goat comprising monosaccharide compositions of GalNAc, Fus, GlcNAc, Gal, Man, and NANA/NGNA; having one of its sites comprising oligomannose and/or hybrid oligosaccharide structures; primarily an oligosaccharide or hybrid type structure on one site and complex oligosaccharide on the remaining three sites; partially sialyated; sialic acid includes NGNA; and fucose on its proximal GlcNAc on each of the sites having complex oligosaccharides. The specification does not reasonably provide enablement for a general model, the applicants are limited to following: transgenic goat for the production of antithrombin III comprising a monosaccharide composition of GalNAc, partially sialyated (NGNA), fucose on its proximal GlcNAc on each f the sites having complex oligosaccharide, and which lacks O-glycosylation, wherein said glycosylation sites comprising oligomannose and/r hybrid structure on one site and complex oligosaccharide on the remaining 3 sites . . .

In view of the quantity of experimentation necessary to determine the parameters listed above, lack of direction or guidance provided by the specification, the absence of working examples for the demonstration of correlation to the production of transgenic animal models of more than one species, or all other glycosylation patterns of antithrombin III, the unpredictable state of the art with respect to the generation of transgenic any and all mammals, it would have

required undue experimentation for one skilled in the art to make and/or use the claimed inventions as broadly claimed.

Applicants respectfully traverse this rejection. Claims 3-5 and 8 have been cancelled, thereby obviating the Examiner's rejection with regard to these claims. The remaining claims, as amended, recite a mammary gland produced ATIII having a particular monosacchride composition, e.g., a monosaccharide composition including GalNAc, which lacks O-linked glycosylation, which is partially sialylated, and/or which has a sialic acid which includes NGNA.

Contrary to the Examiner's assertion, Applicants have provided guidance that transgenic mammals other than goat would produce the claimed monosacchride composition. Applicants have described the production of both transgenic goats and transgenic mice using the same transgene. Both the transgenic goats and the transgenic mice express ATIII having the claimed monosaccharide compositions in their mammary tissue. In particular, at page 8, lines 4-22, Applicants describe the production of transgenic mice by microinjection of the B6C transgene which is the same transgene used to produce the transgenic goats described in the present application. Applicants report expression of ATIII at levels of up to 0.7 to 1.0 mg/ml in the milk of such transgenic mice. Using the same B6C transgene, Applicants also produced transgenic goats which expressed ATIII at levels up to 4 to 6 mg/ml in their milk. In addition, Applicants compared the sialic acid composition of human plasma-derived ATIII, human ATIII produced in the mammary gland of transgenic goats (rhATIII), and human ATIII produced in the milk of transgenic mice (tmATIII). See Figure 7 of the present application. As shown in Figure 7, both human ATIII expressed from transgenic goat mammary glands (rhATIII) and human ATIII expressed from transgenic mouse mammary glands (tmATIII) had a sialic acid composition that included NGNA and NANA, whereas plasma derived ATIII only includes NANA. Thus, Applicants have shown a mammary conditioned as opposed to a species-specific effect. Applicants have further shown that the claimed structures are effectively expressed in the mammary glands in two very different transgenic mammals, goat and mice. When these very different species were microinjected with the same transgene, mammary gland produced ATIII from these mammals had common properties, i.e., the ATIII was partially sialylated and the sialic acid included NGNA.

A further conditioned effect of producing ATIII in the mammary gland as compared to ATIII derived from plasma is shown in Applicants' published work, see, e.g., Cole et al. (1994) J. Cellular Biochemistry Suppl. Vol. 0 (18D) p 265. Applicants reported that the substitution of GalNAc for galactose is the function of expressing ATIII in the mammary gland and not a species difference particular to goat. Since Applicants describe a transgene which encodes ATIII and is expressed in the mammary gland of different species of transgenic mammals, and ATIII produced in the mammary tissue has GalNAc substituted for galactose, there is clearly sufficient guidance to make and use the claimed mammary gland produced ATIII with a monosaccharide composition which includes GalNAc.

Applicants have clearly shown: 1) the generation of two widely divergent transgenic mammals which expresses ATIII in their mammary tissue; 2) the same transgene can be used to produce these different species of transgenic mammals which expresses ATIII; and, 3) ATIII produced in the mammary gland of such mammals has the claimed monosaccharide composition, i.e., a monosaccharide composition including GalNAc, which lacks O-linked glycosylation, which is partially sialylated, and/or which has a sialic acid which includes NGNA. Thus, Applicants have clearly enabled the claimed invention, and therefore, request that the Examiner withdraw this rejection.

Rejection of Claims 1, 2, 7, 8 and 11 under 35 U.S.C. §112, second paragraph

Claims 1, 2, 7, 8 and 11 are rejected under 35 U.S.C. §112, second paragraph, "as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention." In particular, the Examiner states that "claims 1, 2,7, 8, and 11 are vague and indefinite in its recitation of 'includes or including' because it does not define the metes and bounds of the encompassed monosaccharides of the claimed invention."

Claim 8 has been cancelled, thereby obviating the Examiner's rejection of this claim. The remaining claims have been amended to replace the terms "including" or "includes" with the terms "comprising" or "comprises". Thus, Applicants respectfully request that the Examiner withdraw this rejection.

Rejection of Claim 5 Under 35 U.S.C. §102(a)

Claim 5 is rejected under 35 U.S.C. §102(a) as being anticipated by Edmunds et a. 91994) J. Cellular Biochemistry Suppl., Vol. 0 (18D) p. 265. In particular, the Examiner states that

> Edmunds et al. disclosed a detailed oligosaccharide analysis of human plasma ATIII (tgATIII or transgenic ATIII) produced in the milk of transgenic goats and compared the glycosylation found to that of both human and goat plasma derived ATIII (pATIII). The biantennary complex structures were found at 3 of the 4 glycosylation sites in tgATIII with the fourth site (Asn 155) containing High mannose/Hybrid structures (p. 265, U102). Thus, Edmunds clearly anticipates claim 5.

Applicants respectfully request that the Examiner withdraw this rejection in view of the *In re* Katz Declaration by Edward S Cole, Ph.D. submitted on herewith.

Rejection of Claims 1, 4, 6-7 and 10 are rejected under 35 U.S.C. §102(a)

Claims 1, 4, 6, 7, and 10 are rejected under 35 U.S.C. §102(a) as being anticipated by Cole et al. (1994) J. Cellular Biochemistry Suppl. Vol. 0 (18D) p 265. In particular, the Examiner states that

> Cole et al teach the glycosylation patterns and the production of therapeutic proteins (antithrombin III) in the milk of transgenic animals (transgenic goat milk) can be achieved at very high expression levels compared to tissue culture. Cole et al. further discloses the substitution of GalNAc for Gal due to the function of expressing proteins in the mammary gland and not a species difference as goat plasma ATIII does not contain this substitution. The transgenic proteins are more fucosylated and less sialyated than their recombinant or plasma counterparts. NGNA and NANA acid were found on both therapeutic proteins, NGNA therefore appears to be a function of expressing protein in goats (p. 265, U100). Thus, Cole clearly anticipates claims 1, 4, 6-7 and 10.

Applicants respectfully request that the Examiner withdraw this rejection in view of the Second *In re* Katz Declaration by Edward S Cole, Ph.D. submitted on herewith.

Conclusion

Applicant submits that all of the claims are now in condition for allowance, which action is requested. Please apply any other charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: 2 Dec 02

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